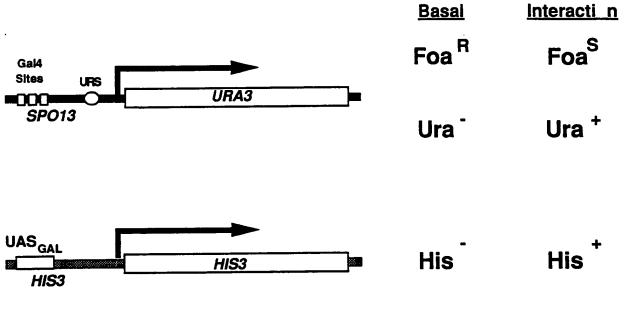
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Applicant(s): Marc Vidal et al.
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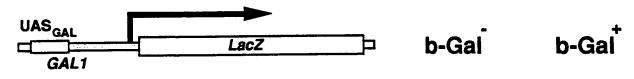


FIG. 1

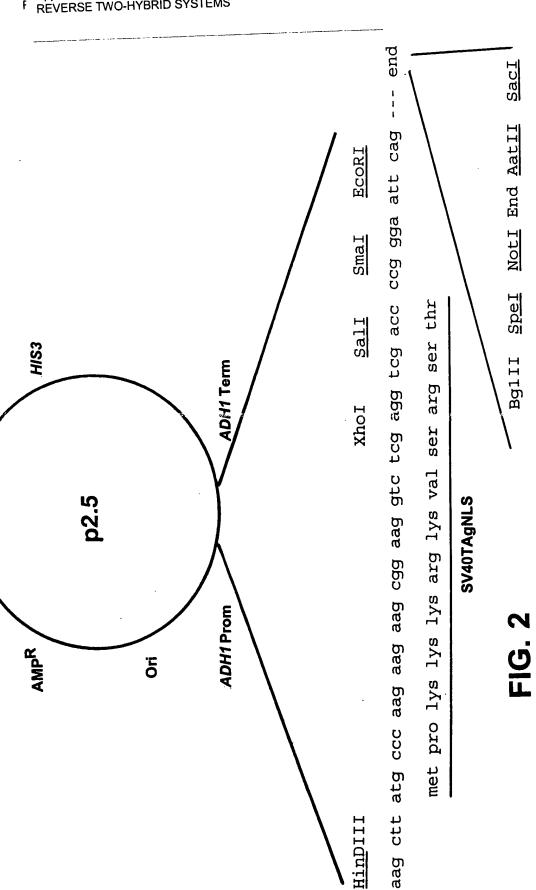
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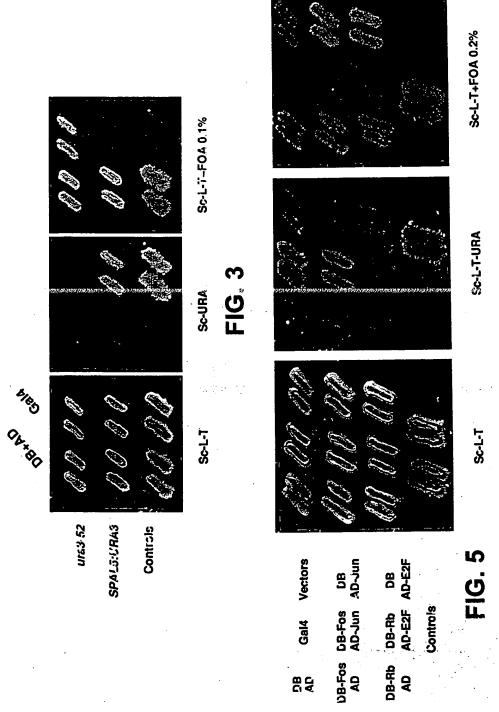
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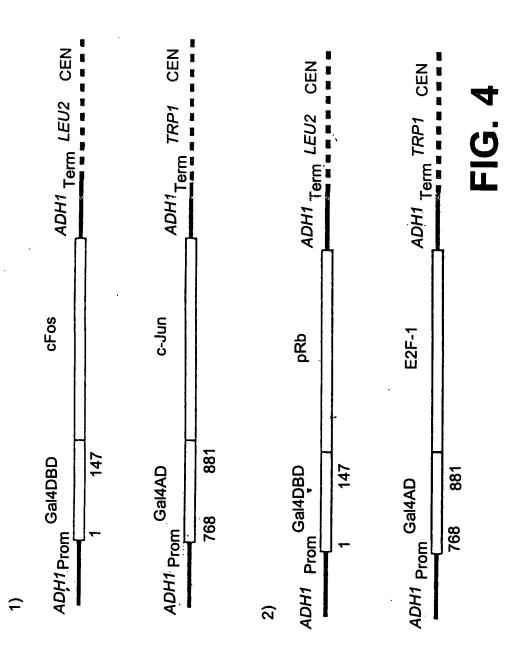






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Applicant(s): Marc Vidal et al.
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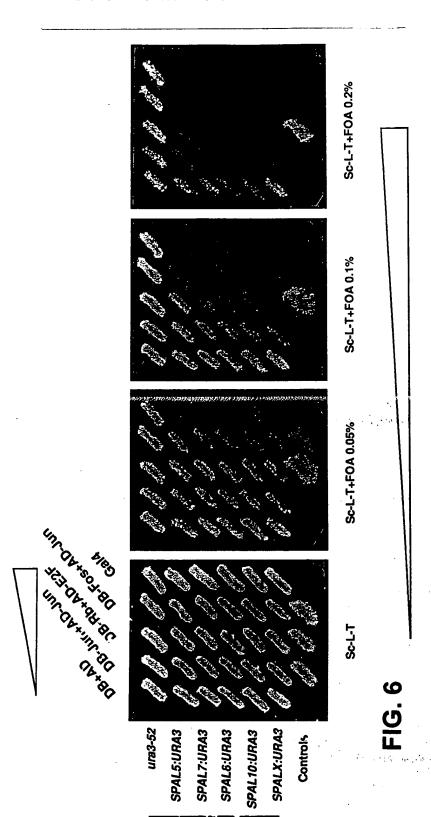
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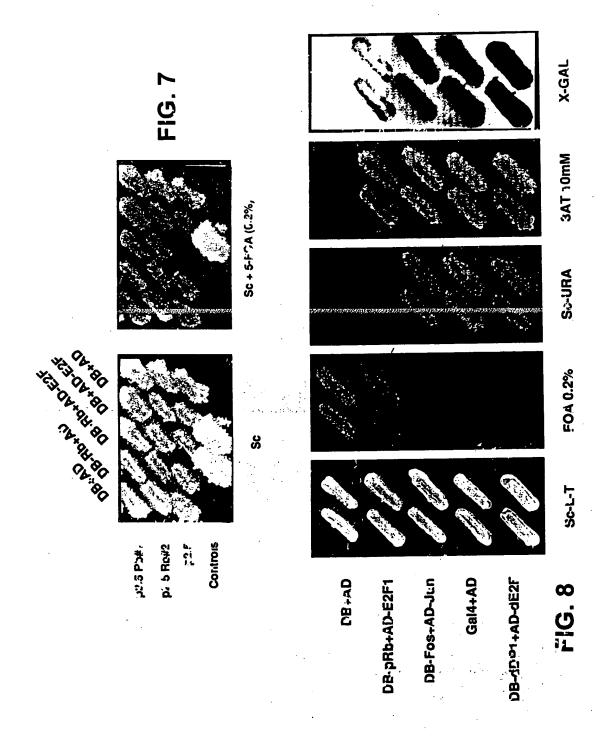
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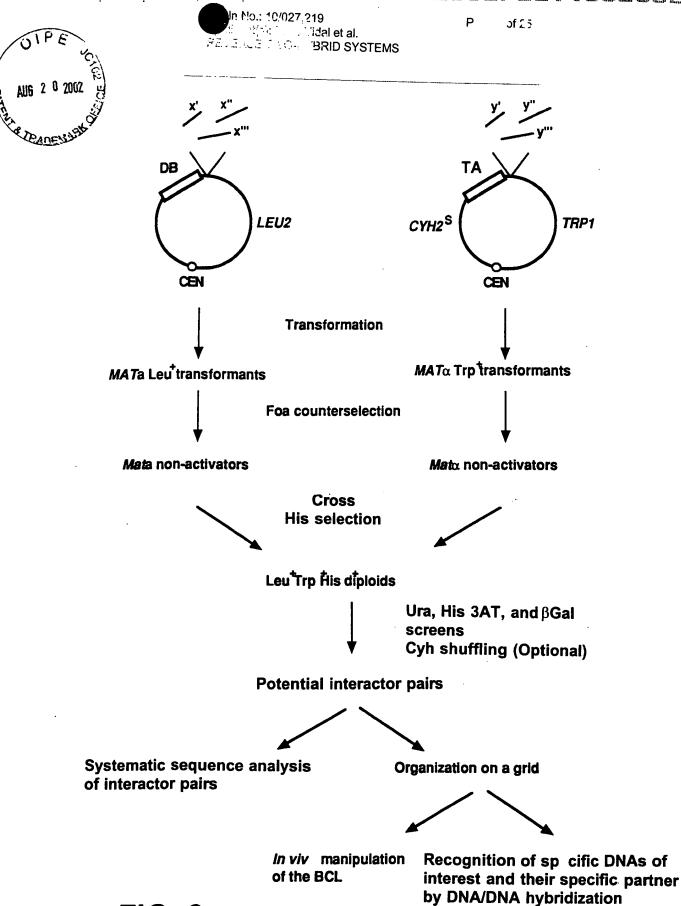
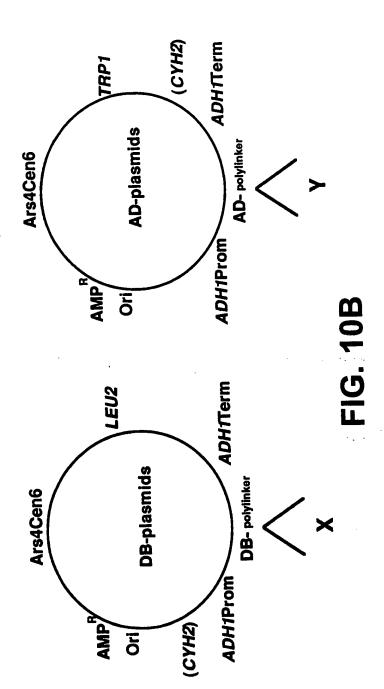


FIG. 9

n No.: 10/027,219 ⊝an'(s): Clarc Vidal et al. ERSE TWU-HYBRID SYSTEMS AUG 2 0 2002 H -Xbal T_{ADCI} GAL4 880 881 1xs glu gly ser thr pro gly 11e gln 11e tyr. end Ara gag cgt ggg tog ogg att cag atc tag tgcggccgg -EcoRV Not I Hind III -BamH -Pvu II Sac II -Pvu I ARSH4 TRPI CEN6 -Sph I Sma I EcoR I Bgl II T_{ADCI} GAL4(TA) Amp^r Pvu I-J NLS Aat II Sac I PADCI ColE1 Sal I on Xho I-Hind III Sph I-EcoRV. Nar IX Pvu II-Apa I Kpn I **EcoRV** /EcoRI -Cla I -Kpn I T_{ADCI} GAL4
146 147
VAL SER ARG SER THR PRO GLY ZLE GLN ILE TYR END
GTA TCG TCG AGG TCG ACC CCG GGA ATT CAG ATC TAC TAG TGCGGCCGC END END END
TAAGTAAGAAGACGCCGAAGGGAGGATTT
TAAGTAAGAAGAAGGGCCGCCACGGGGGGGAGGTTT Not I -Nar I Sac II Spe I LEU2 -BamH I ARSH4 Pvu I -Sph I Sma I EcoR I Bel II T_{ADCI} CEN6 GAL4(DB) Amp' Aat II Sac I PADCI Sal I Xho I Sph I Hind III-ColE1 Pvu I 07. Sph I EcoRV-Nar I-Xho 1-Kpn I-Pvu II-Apa 1

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REVERSE TWO-HYBRID SYSTEMS



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(3)			Applin No.: 10/027,219 Applicant(s): Marc Vidal et al. REVERSE TWO-HYBRID SYSTEMS					Page 10 of 25			
2 0 2002 H											
False positive	·				0						
"Novel" interacting		2	<u>†</u>		8 + 5						
Known interacting		0	6		0			0		FIG. 11	
Retested	0	o,	7	0	©	16	16	ន	23		
His+	-	6	_	8	8	8	88	8	8		
Total	1x10 ⁶	5x10 ⁵	2x10 ⁵	1x10 ⁶	1x10 ⁶	1x10 ⁶	1x10 ⁶	3x10 ⁶	1x10 ⁶		
DB-X	None	p130	DP1	pRb	p35	СБКЗ	СОКЗ	DCC1	nq z		



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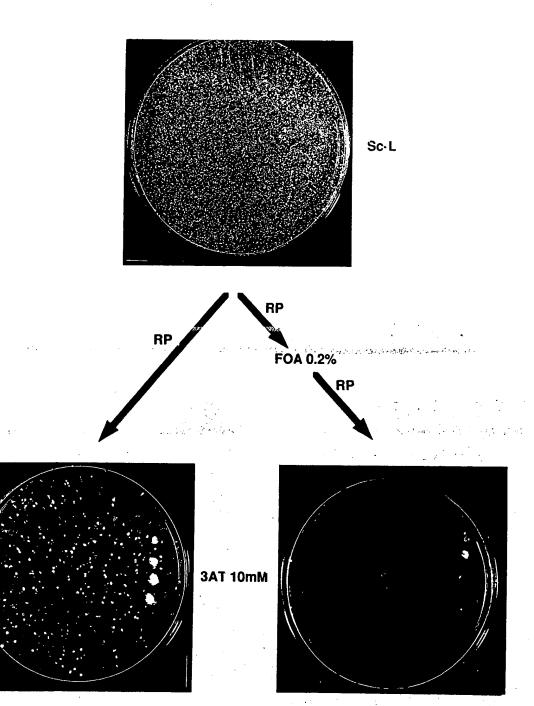
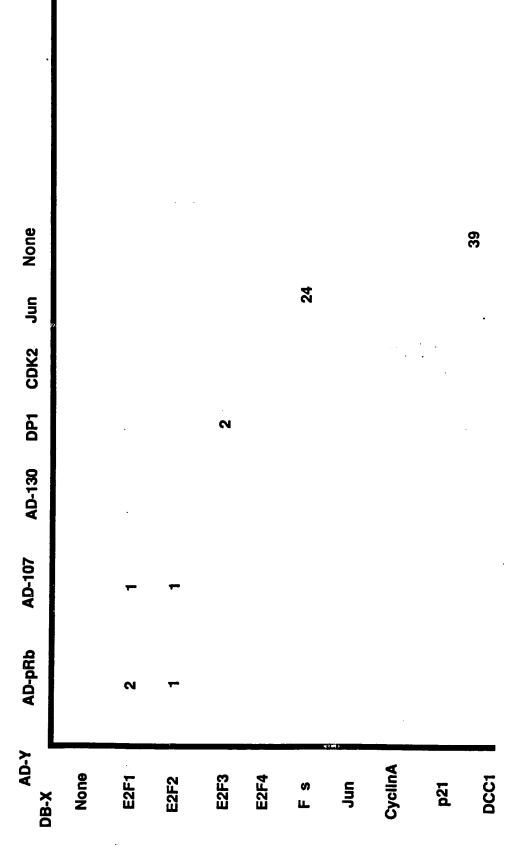


FIG. 12

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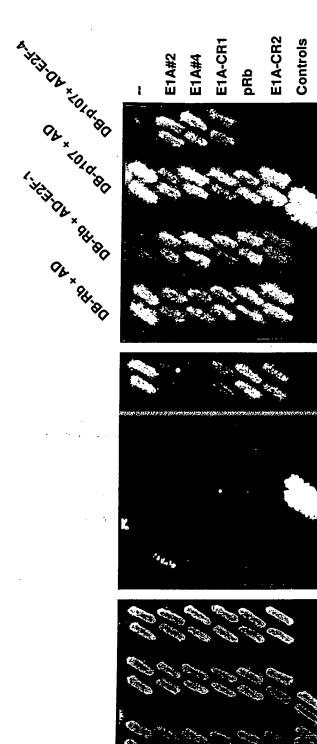


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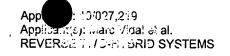
Appln No.: 10/027,219 Applicant(s): Marc Vidal et al. REVERSE TWO-HYBRID SYSTEMS



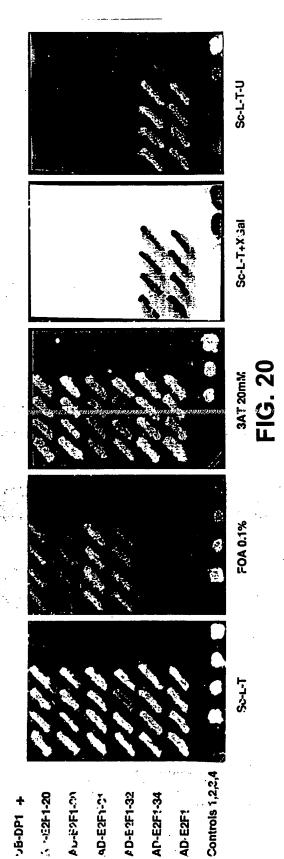


Sc -L-T-H+FOA 0.2%

Sc-L-T-H-U



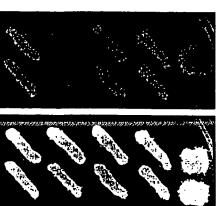




AP-E2F1-34

AD E2F!

FIG. 15



DE-Rba22+AD-E2F1

DB+AD

DB-Rb+AD-E2F1

Sc-L-T+FOA 0.2%

Sr-L-T

Controls

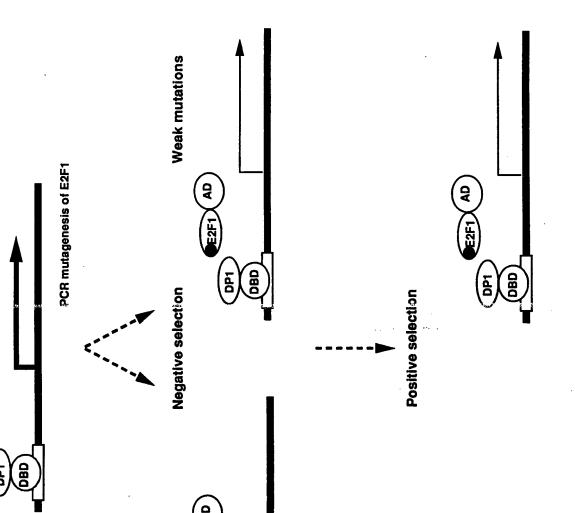
Db-F ... AU-E2FY411C

.√6-DP1 +

∴ --122F-1-20

AD-E2F1-34

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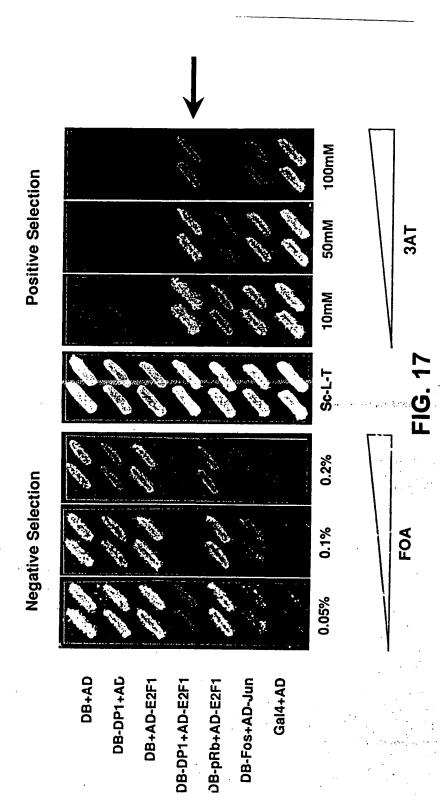
Strong mutations

FIG. 16

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PCR reaction

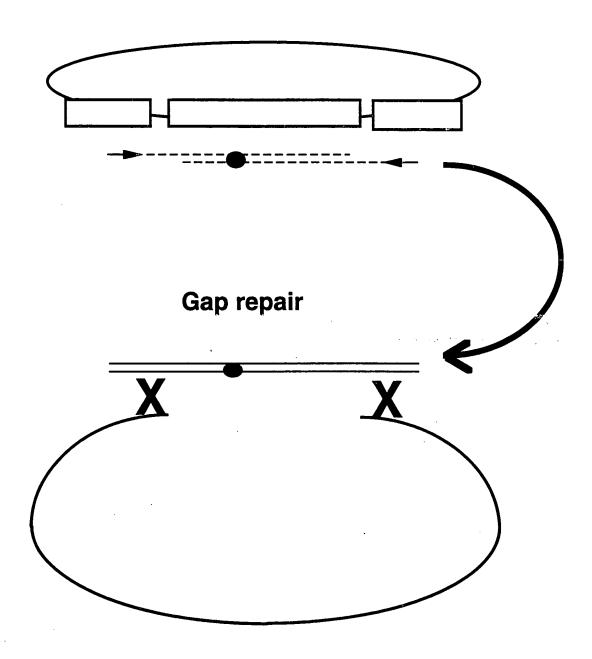


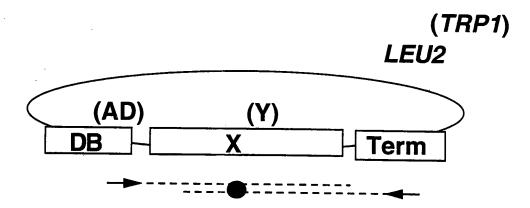
FIG. 18A



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In vitro mutagenic PCR reaction



In vivo gap repair

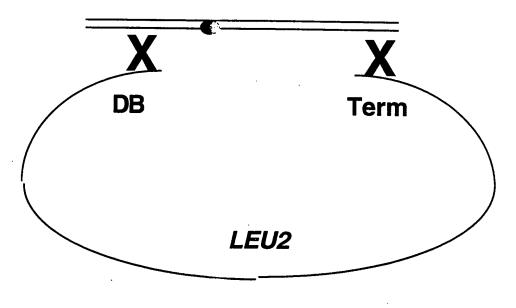
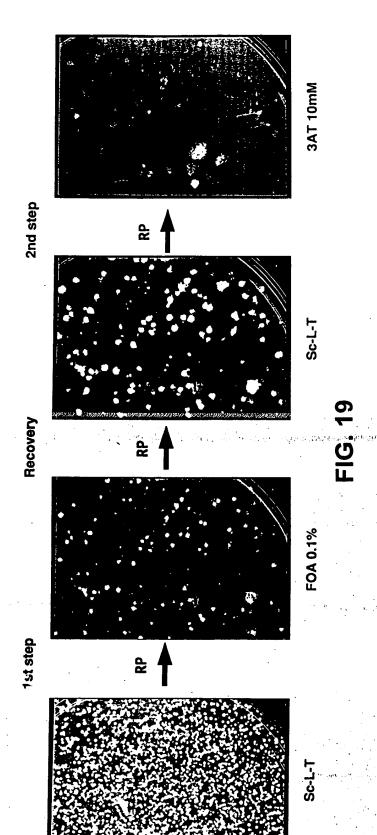


FIG. 18B

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MARKED BOX 2

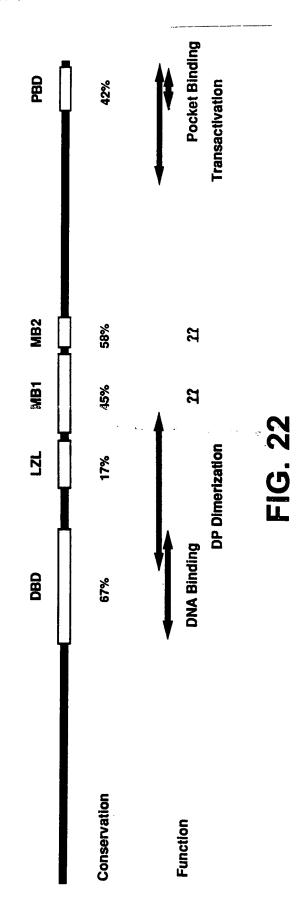
	20	30	32	31	65
E2F5 E2F4 E2F3 E2F2 E2F2	E2F1-;	E2F1-	E2F1-	E2F1-	E2F1-
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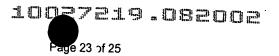
Appln No.: 10/027,219
Applicant(s): Marc Vidal et al.
REVERSE TWO-HYBRID SYSTEMS

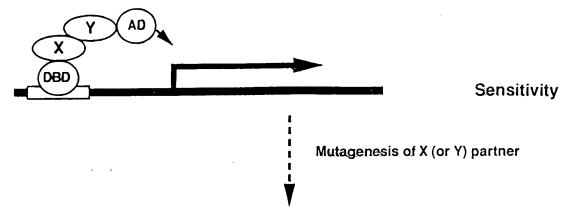
MAT a Trp+ Cyhs Leu+ transformants MAT & Trp+ Cyhs Leu+ non-interactors MAT a Trp- Cyhr Leu+ non-interactors MATa Trp+ Cyhs transformants His 3AT positive selection PCR mutagenesis of the reference sequence Introduction of wild-type shuffle plasmids into haploid cells of different mating-type haploid cells of different mating-type and gap repair into corresponding **Compensatory mutations** Foa counterselection Leu+ Trp+ diploids Plasmid shuffling Mass mating MATa Leu+ Cyhs Trp+ non-interactors MATa Leu+ Cyhs Trp+ transformants MATa Leu- Cyhr Trp+ non-interactors MATa Leu+ Cyhs transformants

FIG. 23A

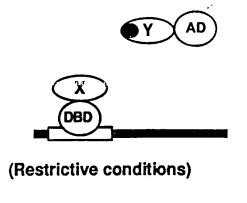


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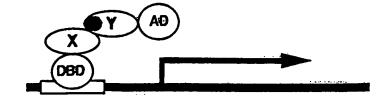






SELECTION for Ura/His growth

FIG. 23B



(Permissive conditions)

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Restrictive Conditions Permissive Conditions 36C 30C Sc+5-FOA (0.2%) Sc Stronger Phenotypes DB+AD DB-Fos+AD-Jun Controls